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Comparison of (1→3)-β-D-Glucan, Mannan/Anti-Mannan Antibodies, and Cand-Tec *Candida* Antigen as Serum Biomarkers for Candidemia

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We conducted a case-control study using the Fungitell assay, the novel Platelia *Candida* Antigen (Ag) Plus and *Candida* Antibody (Ab) Plus assays, and the Cand-Tec latex agglutination test to evaluate the usefulness of (1→3)-β-D-glucan (BDG), mannan antigen with/without anti-mannan antibody, and Cand-Tec *Candida* antigen measurement for the diagnosis of candidemia. A total of 56 patients fulfilled the inclusion criteria and were enrolled. One hundred patients with bacteremia and 100 patients with sterile blood cultures served as negative controls. In the candidemia group, median (1→3)-β-D-glucan, mannan antigen, and anti-mannan antibody levels were 427 pg/ml, 190 pg/ml, and 18.6 antibody units (AU)/ml, respectively. All three parameters were significantly elevated in patients with candidemia. The sensitivity and specificity were, respectively, 87.5% and 85.5% for (1→3)-β-D-glucan, 58.9% and 97.5% for mannan antigen, 62.5% and 65.0% for anti-mannan antibody, 89.3% and 63.0% for mannan antigen plus anti-mannan antibody, 89.3% and 85.0% for BDG plus mannan antigen, and 13.0% and 93.9% for Cand-Tec *Candida* antigen. The low mannan antigen sensitivity was in part caused by *Candida parapsilosis* and *Candida guilliermondii* fungemias, which were not detected by the Platelia *Candida* Ag Plus assay. When the cutoff was lowered from 125 pg/ml to 50 pg/ml, mannan antigen sensitivity increased to 69.6% without severely affecting the specificity (93.5%). Contrary to recently published data, superficial candidiasis was not associated with elevated mannan antigen levels, not even after the cutoff was lowered. Combining procalcitonin (PCT) with (1→3)-β-D-glucan to increase specificity provided a limited advantage because the benefit of the combination did not outweigh the loss of sensitivity. Our results demonstrate that the Cand-Tec *Candida* antigen and the mannan antigen plus anti-mannan antibody measurements have unacceptably low sensitivity or specificity. Of the four tests compared, (1→3)-β-D-glucan and mannan antigen are the superior biomarkers, depending on whether a sensitivity-driven or specificity-driven approach is used.

Candida species account for approximately 10% of bloodstream infections (BSI) in intensive care units (ICUs) and are associated with a crude in-hospital mortality rate of 30% (1, 2). Because a delay in the initiation of antimycotic therapy is associated with increased mortality, timely diagnosis is of utmost importance (3). While blood cultures are still regarded as the gold standard for diagnosis of candidemia, it takes about 2 days to obtain a positive result, and sensitivity can be as low as 50% (4, 5). With these limitations of culture-based *Candida* detection, it is quite evident that faster and more sensitive techniques are required.

For this purpose a number of clinical prediction rules (6–8) and non-culture-based methods are available. The latter include the detection of *Candida* DNA and circulating fungal antigens in serum. While nucleic acid amplification techniques are still lacking standardization, commercial tests are available for the measurement of (1→3)-β-D-glucan (BDG), mannan antigen (Ag), and Cand-Tec *Candida* antigen (CA). All of these tests have been evaluated for their performance in the diagnosis of invasive candidiasis with sensitivities and specificities, respectively, of 77% and 85% for BDG, 58% and 93% for mannan Ag, and 64% and 58% for CA (9). By combining mannan Ag with anti-mannan antibody (Ab) measurement, the sensitivity and specificity can be increased to 83% and 86%, respectively (10). Recently, there have been changes concerning these biomarkers. It has been reported that BDG levels are elevated in bacteremia, questioning the validity of this marker for the diagnosis of invasive fungal disease (11, 12). However, studies including relevant numbers of bacteremic patients are lacking. Furthermore, the most widely used assay for mannan Ag detection, the Platelia *Candida* Ag assay, was recently refined (now known as the Platelia *Candida* Ag Plus assay). So far, only one study has examined this novel test format (13).

We therefore conducted a case-control study to compare the diagnostic performance of serum BDG (Fungitell), mannan Ag with/without mannan Ab (Platelia *Candida* Ag/Ab Plus), and CA (Cand-Tec) detection for the diagnosis of candidemia.

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MATERIALS AND METHODS

Candidemia patients. All patients presenting at the University Medical Centre Freiburg, Germany, between January 2001 and May 2012 were enrolled. Inclusion criteria were a culture-confirmed candidemia and an archived serum sample from day 0 until day 2 after blood culture sampling. An exclusion criterion was treatment with intravenous immunoglobulins (IVIG) or albumin in the 7 days prior to serum sampling. Patients receiving IVIG and albumin were excluded because we have found in the past that, without exception, even a single administration of these

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TABLE 1 Baseline characteristics of the study populations and risk factors for candidemia

Parameter[b]	Value for the parameter by patient group[a]		
	Control group 1	Control group 2	Candidemia group[g]
No. of patients	100	100	56
Median age (yr [IQR])	57 (45–67)	61 (48–73)	61 (49–71)
Sex (no. of males/no. of females)	66/34	65/35	28/28
% of patients in an ICU (yes/no)	7 (7/93)	17 (17/83)	39 (22/34)
Median no. of days in an ICU before blood culture sampling (range)	3 (1–8)	2 (1–116)	7.5 (1–49)
% of patients with a central venous catheter (yes/no)	37 (37/63)	43 (43/57)	80 (45/11)
No. of patients with a central venous catheter			
Nontunneled	31	34	28
Tunneled	1	0	2
Port	5	9	18
% of patients with *Candida* colonization or infection (yes/no)[c]	19 (19/81)	20 (20/80)	50 (28/28)
No. of patients with *Candida* colonization at:			
1 site	16	20	15
2 sites	3	0	12
More than 2 sites	0	0	1
% of patients with a history of:			
Major surgery prior to blood culture sampling (yes/no)[d]	5 (5/95)	6 (6/94)	25 (14/42)
Major abdominal surgery prior to blood culture sampling (yes/no)[d]	1 (1/99)	4 (4/96)	16 (9/47)
Immunosuppression (yes/no)	55 (55/45)	57 (57/43)	52 (29/27)
Dialysis (yes/no)[e]	1 (1/99)	4 (4/96)	16 (9/47)
Broad spectrum antibiotic therapy (yes/no)[f]	68 (68/32)	57 (57/43)	79 (44/12)

[a] Control group 1, negative blood culture; control group 2, bacteremia.
[b] IQR, interquartile range; ICU, intensive care unit; yes/no, number of patients positive for the characteristic/number of patients negative for the characteristic.
[c] Positive culture for *Candida* species from any nonsterile body site.
[d] Surgery during the 4 weeks prior to blood culture sampling.
[e] Dialysis membranes used at the University Medical Centre Freiburg do not influence BDG levels (data not shown).
[f] Therapy during the 7 days prior to blood culture sampling.
[g] Candidemia risk factors are more prevalent in the candidemia group (e.g., length of stay in an ICU, existence of central venous catheters, *Candida* colonization/superficial infection, dialysis, broad-spectrum antibiotic therapy).

substances causes significantly elevated BDG levels that usually normalize within 1 week (14).

A total of 79,840 blood cultures were examined. *Candida* species were detected in 754 of 10,987 positive blood cultures (6.9%) from 307 patients. Sixty-eight patients fulfilled the inclusion criteria. Twelve patients were excluded because they had received IVIG or albumin. Finally, 56 patients were enrolled in the study. The underlying diseases were hematologic malignancy/hematopoietic stem cell transplantation (HSCT; $n = 9$), solid tumor ($n = 12$), gastrointestinal disease ($n = 6$), major surgery ($n = 4$), abdominal surgery ($n = 16$), and miscellaneous disease ($n = 9$). The *Candida* species isolated were *Candida albicans* ($n = 32$), *Candida glabrata* ($n = 11$), *Candida tropicalis* ($n = 6$), *Candida parapsilosis* ($n = 4$), and *Candida guilliermondii* ($n = 3$).

Controls. Sera from two control groups were examined. Control group 1 consisted of 100 patients with a negative blood culture, and control group 2 consisted of 100 patients with bacteremia. In both groups an archived serum sample from the day of blood culture sampling was required for inclusion. Control patients receiving IVIG or albumin were excluded. Bacteremia was caused by the following pathogens: *Bacillus* species ($n = 3$), *Bacteroides* species ($n = 3$), *Citrobacter* species ($n = 2$), *Enterobacter cloacae* ($n = 5$), *Enterococcus faecalis* ($n = 5$), *Enterococcus faecium* ($n = 5$), *Escherichia coli* ($n = 5$), *Haemophilus influenzae* ($n = 2$), *Klebsiella oxytoca* ($n = 3$), *Klebsiella pneumoniae* ($n = 3$), *Listeria monocytogenes* ($n = 2$), *Pseudomonas aeruginosa* ($n = 10$), *Serratia marcescens* ($n = 3$), *Staphylococcus aureus* ($n = 10$), *Staphylococcus epidermidis* ($n = 5$), *Staphylococcus haemolyticus* ($n = 3$), *Staphylococcus hominis* ($n = 5$), *Streptococcus agalactiae* ($n = 3$), *Streptococcus anginosus* group ($n = 3$), *Streptococcus bovis* ($n = 3$), *Streptococcus dysgalactiae* ($n = 2$), *Streptococcus mitis* ($n = 3$), *Streptococcus mutans* ($n = 2$), *Streptococcus oralis* ($n = 2$), *Streptococcus pneumoniae* ($n = 5$), and *Streptococcus pyogenes* ($n = 3$). Patients with bacteremia caused by coagulase-negative staphylococci were included only if at least two different blood culture sets were positive.

Serum and data collection. Patient demographics, clinical characteristics, laboratory results, risk factors for candidemia, and microbiological results were collected (Table 1). Serum samples were originally taken for various microbiological analyses and were frozen at −80°C. The use of these sera was approved by the local ethics committee (application number 293/11).

Serum antigen measurement. All tests were performed according to the manufacturer's recommendations at our own institution. The persons who tested the sera were not blinded. The Fungitell assay (Associates of Cape Cod, MA) was used for BDG measurement. The recommended cutoff is ≥80 pg/ml. Each serum was tested in duplicate. Samples with BDG levels above 500 pg/ml were diluted and retested. BDG levels below 31 pg/ml (lower validation limit) were calculated by extrapolation.

The Platelia *Candida* Ag Plus and the Platelia *Candida* Ab Plus assays (Bio-Rad, France) were used for mannan Ag and mannan Ab measurement, respectively. The recommended cutoff is ≥125 pg/ml for the antigen and ≥10 antibody units (AU)/ml for the antibody. Samples with mannan Ag levels above 500 pg/ml were diluted and retested.

The Cand-Tec latex agglutination test (Ramco Laboratories, TX) was used for CA measurement. The recommended cutoff titer is ≥1:4. Each CA test was checked by two readers, and if the results were incongruent, they were ratified by a third user in a blinded fashion. CA-positive sera were not titrated to the endpoint, and therefore the results are only qualitative.

All assays used bear the European CE marking. The Fungitell assay in addition is FDA approved.

Statistical methods. Statistical analysis was performed using SPSS, version 19, and MedCalc, version 12. For the comparison of variables, Fisher's exact test, a Mann-Whitney U test, and a Kruskal-Wallis test were

TABLE 2 Main test results stratified according to patient group

Parameter and/or interpretation method[d]	Value for the parameter by patient group[a]		
	Control group 1	Control group 2	Candidemia group
Mean time span from blood culture to serum sampling (days [range])	0	0	0.5 (0–2)
Median BDG concn (pg/ml [IQR])	26 (9–46)	17 (0–59)	427 (133–985)
Median mannan Ag concn (pg/ml [IQR])	3 (0–12)	0 (0–14)	190 (22–801)
Median mannan Ab concn (AU/ml [IQR])	1.5 (0–18.2)	0.5 (0–23.3)	18.6 (1.2–42.7)
Test interpretation by:			
Manufacturer's cutoffs (no. of patients positive/indeterminate/negative)[b]			
BDG	10/4/86	19/6/75	49/1/6
Mannan Ag	2/1/97	3/4/93	33/4/19
Mannan Ab	34/8/58	36/9/55	35/4/17
Mannan Ag + Ab	35/7/58	39/13/48	50/1/5
CA	1/99	11/85	7/47
Optimized cutoffs (no. of patients positive/negative)[c]			
BDG	11/89	21/79	50/6
Mannan Ag	5/95	8/92	39/17
Mannan Ab	28/72	29/71	33/23
Mannan Ag + Ab	32/68	37/63	50/6

[a] Control group 1, negative blood culture; control group 2, bacteremia.
[b] BDG manufacturer's cutoffs: positive, ≥80 pg/ml; indeterminate, 60 to 79 pg/ml; negative <60 pg/ml; Mannan Ag cutoffs: positive, ≥125 pg/ml; intermediate, 62.5 to 125 pg/ml; negative, <62.5 pg/ml. Mannan Ab cutoffs: positive ≥10 AU/ml; intermediate, 5 to 10 AU/ml; negative, <5 AU/ml. CA results: positive, ≥1:4; negative, <1:4.
[c] BDG optimized cutoffs: positive, ≥70 pg/ml; negative, <70 pg/ml. Mannan Ag cutoffs: positive, >50 pg/ml; negative, ≤50 pg/ml. Mannan Ab cutoffs: positive, ≥15 AU/ml; negative, <15 AU/ml. No optimized cutoff was determined for CA.
[d] BDG, (1→3)-β-D-glucan; IQR, interquartile range; Ag, antigen; Ab, antibody; AU, antibody unit; CA, *Candida* antigen.

used. Differences were considered significant at a P value of <0.05. The optimal cutoffs were determined by receiver operating characteristic (ROC) analysis (maximum Youden index).

RESULTS

Diagnostic performance. The main results are shown in Tables 2 and 3. In the candidemia group, median analyte levels were 427 pg/ml (BDG), 190 pg/ml (mannan Ag), and 18.6 AU/ml (mannan Ab). All three parameters were significantly elevated in patients with candidemia ($P < 0.001$). To determine optimized cutoffs, ROC analyses were performed. The ROC curves are depicted in Fig. 1. As mentioned in Materials and Methods, CA results are only qualitative, and therefore no ROC analysis was performed. The area under the ROC curve (AUC) was largest for BDG (0.925); however, the difference between the AUCs of BDG and mannan Ag (0.898) was statistically not significant ($P = 0.361$). The AUCs of both BDG and mannan Ag were significantly larger than the AUC of mannan Ab (0.673; $P < 0.001$). The optimized cutoffs were ≥70 pg/ml for BDG, >50 pg/ml for mannan Ag, and ≥15 AU/ml for mannan Ab.

The sensitivities using the manufacturer's and the optimized cutoffs, respectively, were 87.5% versus 89.3% for BDG, 58.9% versus 69.6% for mannan Ag, 62.5% versus 58.9% for mannan Ab, 89.3% versus 89.3% for mannan Ag plus Ab, and 89.3% versus 92.9% for BDG plus mannan Ag. The overall specificities, again listed for the manufacturer's and the optimized cutoffs, were 85.5% versus 84.0% for BDG, 97.5% versus 93.5% for mannan Ag, 65.0% versus 71.5% for mannan Ab, 63.0% versus 65.5% for mannan Ag plus Ab, and 85.0% versus 81.0% for BDG plus mannan Ag. No optimized cutoffs were determined for CA; using the manufacturer's cutoffs sensitivity was 13.0% and specificity was 93.9% for CA. The specificity of BDG in patients with bacteremia (81%) was lower than in patients with negative blood cultures (90%; $P = 0.053$). The specificities of the other analytes were independent of the control group.

Combination of BDG with PCT to increase specificity. Procalcitonin (PCT) was measured in 29 patients with candidemia (mean PCT of 0.80 μg/liter), 52 patients with bacteremia (mean PCT of 2.36 μg/liter), and 52 patients with negative blood cultures (mean PCT of 0.27 μg/liter). Patients with bacteremia had significantly higher PCT levels than the combined group consisting of patients with candidemia plus negative blood cultures ($P = 0.001$). A recommended cutoff for discriminating these two patient populations is a PCT value of ≥2.0 μg/liter (15). The combination of BDG with PCT led to a considerable increase in specificity from 89.4% to 96.2%, accompanied by a loss of sensitivity from 86.7% to 51.7%.

BDG and mannan Ag in microbiological and clinical subgroups. Subgroup analysis of BDG and mannan Ag (Table 4) revealed that both parameters were significantly elevated in control patients following major surgery and abdominal surgery during the 4 weeks prior to serum sampling ($P = 0.009$ and $P = 0.010$ for BDG and mannan AG, respectively). While median BDG and mannan Ag levels in patients after major surgery were both below the cutoff, the median BDG level after abdominal surgery was 174 pg/ml and therefore would lead to a positive test interpretation. As mentioned above, the BDG false-positivity rate was higher in patients with bacteremia than in patients with negative blood cultures (19% versus 10%; $P = 0.053$). While there was no difference in BDG levels between Gram-positive and Gram-negative BSI ($P = 0.76$), patients with *Enterococcus faecalis* bacteremia had significantly higher BDG levels than patients with bacteremia of other origins (135 pg/ml versus 15 pg/ml; $P = 0.04$). Raising the BDG cutoff to 135 pg/ml resulted in an overall sensitivity and specificity of 77.0% and 89.5%, respectively.

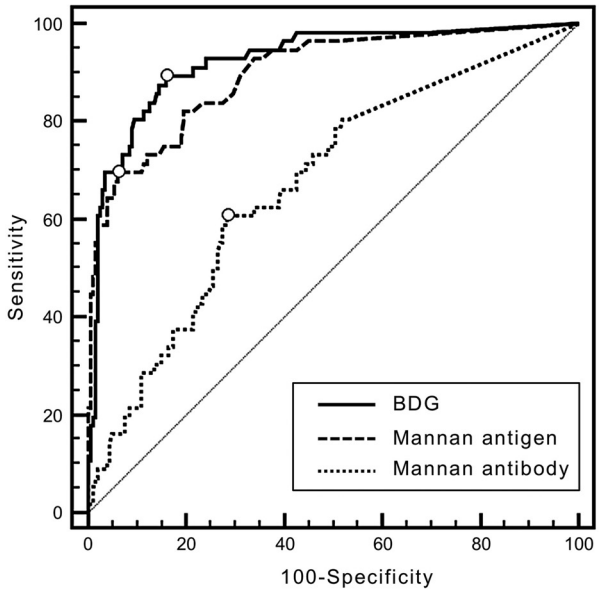
TABLE 3 Diagnostic performance of the different biomarkers using manufacturer and optimized cutoffs

Diagnostic parameter[a]	Value for the indicated marker(s)					
	BDG	Mannan Ag	Mannan Ab	CA	Mannan Ag + Ab	BDG + mannan Ag
Manufacturer's cutoffs						
Sensitivity (% [95% CI])	87.5 (75.9–94.8)	58.9 (45.0–71.9)	62.5 (48.6–75.1)	13.0 (5.4–24.9)	89.3 (78.1–95.9)	89.3 (78.1–95.9)
Specificity (% [95% CI])[b]						
Negative blood culture	90.0 (82.4–95.1)	98.0 (93.0–99.7)	66.0 (55.9–75.2)	99.0 (94.5–99.8)	65.0 (54.7–74.1)	90.0 (82.4–95.1)
Bacteremia	81.0 (71.9–88.2)	97.0 (91.5–99.3)	64.0 (53.8–73.4)	88.5 (80.4–94.1)	61.0 (50.7–70.6)	80.0 (70.8–87.3)
Overall	85.5 (79.8–90.1)	97.5 (94.3–99.2)	65.0 (58.0–71.6)	93.9 (89.6–96.8)	63.0 (55.9–69.7)	85.0 (79.3–89.6)
Positive likelihood ratio (95% CI)[b]						
Negative blood culture	8.75 (4.82–15.88)	29.46 (7.34–118.21)	1.84 (1.31–2.58)	12.96 (1.64–102.63)	2.55 (1.92–3.38)	8.93 (4.92–16.19)
Bacteremia	4.61 (3.04–6.99)	19.64 (6.31–61.16)	1.74 (1.25–2.42)	1.13 (0.47–2.75)	2.29 (1.76–2.97)	4.46 (2.99–6.68)
Overall	6.03 (4.25–8.57)	23.57 (9.65–57.56)	1.79 (1.35–2.36)	2.12 (0.88–5.12)	2.41 (1.97–2.95)	5.95 (4.23–8.38)
Negative likelihood ratio (95% CI)[b]						
Negative blood culture	0.14 (0.07–0.28)	0.42 (0.31–0.57)	0.57 (0.39–0.82)	0.88 (0.79–0.98)	0.16 (0.08–0.36)	0.12 (0.06–0.25)
Bacteremia	0.15 (0.08–0.31)	0.42 (0.31–0.58)	0.59 (0.41–0.85)	0.98 (0.87–1.11)	0.18 (0.08–0.38)	0.13 (0.06–0.29)
Overall	0.15 (0.07–0.29)	0.42 (0.31–0.58)	0.58 (0.41–0.82)	0.93 (0.83–1.03)	0.17 (0.08–0.36)	0.13 (0.06–0.27)
Positive predictive value (% [95% CI])[c]						
All patients with blood culture	6.4 (1.0–19.3)	20.6 (1.4–64.0)	1.9 (0.2–7.3)	2.3 (0.0–25.6)	2.6 (0.4–8.1)	6.2 (1.0–18.6)
Patients with BSI	37.9 (24.9–52.2)	70.0 (45.0–88.5)	15.0 (8.5–23.8)	17.4 (3.7–43.1)	19.3 (12.3–28.1)	37.1 (24.4–51.1)
ICU patients with BSI	40.9 (27.8–55.0)	72.6 (48.4–89.8)	16.7 (9.9–25.7)	19.3 (4.6–45.3)	21.3 (14.0–30.3)	40.1 (27.3–53.9)
Negative predictive value (% [95% CI])[c]						
All patients with blood culture	99.8 (98.0–100)	99.5 (97.8–100)	99.4 (96.6–100)	99.0 (96.7–99.8)	99.8 (97.3–100)	99.9 (98.0–100)
Patients with BSI	98.6 (95.8–99.7)	96.0 (92.6–98.1)	94.6 (89.9–97.6)	91.6 (87.3–94.8)	98.3 (94.8–99.7)	98.8 (96.1–99.8)
ICU patients with BSI	98.4 (95.5–99.6)	95.5 (92.0–97.8)	93.9 (89.0–97.1)	90.6 (86.1–94.0)	98.1 (94.4–99.7)	98.6 (95.8–99.7)
Optimized cutoffs						
Sensitivity (% [95% CI])	89.3 (78.1–95.9)	69.6 (55.9–81.2)	58.9 (45.0–71.9)		89.3 (78.1–95.9)	92.9 (82.7–98.0)
Specificity (% [95% CI])[b]						
Negative blood culture	89.0 (81.2–94.4)	95.0 (88.7–98.3)	72.0 (62.1–80.5)		68.0 (57.9–77.0)	87.0 (78.8–92.9)
Bacteremia	79.0 (69.7–86.5)	92.0 (84.8–96.5)	71.0 (61.1–79.6)		63.0 (52.8–72.4)	75.0 (65.3–83.1)
Overall	84.0 (78.2–88.8)	93.5 (89.1–96.5)	71.5 (64.7–77.6)		65.5 (58.5–72.1)	81.0 (74.9–82.2)
Positive likelihood ratio (95% CI)[b]						
Negative blood culture	8.12 (4.61–14.28)	13.93 (5.83–33.30)	2.10 (1.44–3.09)		2.79 (2.07–3.77)	7.14 (4.28–11.92)
Bacteremia	4.25 (2.88–6.28)	8.71 (4.38–17.30)	2.03 (1.39–2.96)		2.41 (1.84–3.17)	3.71 (2.62–5.26)
Overall	5.58 (4.01–7.76)	10.71 (6.16–18.63)	2.07 (1.52–2.82)		2.59 (2.09–3.20)	4.89 (3.64–6.57)
Negative likelihood ratio (95% CI)[b]						
Negative blood culture	0.12 (0.06–0.26)	0.32 (0.21–0.48)	0.57 (0.41–0.80)		0.16 (0.07–0.34)	0.08 (0.03–0.21)
Bacteremia	0.14 (0.06–0.29)	0.33 (0.22–0.49)	0.58 (0.41–0.81)		0.17 (0.08–0.37)	0.10 (0.04–0.25)
Overall	0.13 (0.06–0.27)	0.32 (0.22–0.48)	0.57 (0.41–0.80)		0.16 (0.08–0.35)	0.09 (0.03–0.23)
Positive predictive value (% [95% CI])[c]						
All patients with blood culture	5.8 (1.0–17.4)	10.6 (1.2–34.0)	2.2 (0.2–8.8)		2.8 (0.5–8.7)	5.1 (0.9–15.3)
Patients with BSI	35.6 (23.3–49.4)	51.4 (32.9–69.7)	17.0 (9.5–27.1)		20.4 (13.0–29.6)	32.6 (21.5–45.6)
ICU patients with BSI	38.5 (26.2–52.1)	54.6 (36.1–72.2)	18.8 (11.0–29.1)		22.5 (14.9–31.9)	35.5 (24.2–48.1)
Negative predictive value (% [95% CI])[c]						
All patients with blood culture	99.9 (98.0–100)	99.6 (97.8–100)	99.4 (96.8–100.0)		99.8 (97.4–100)	99.9 (98.0–100)
Patients with BSI	98.8 (96.0–99.8)	96.9 (93.7–98.7)	94.6 (90.2–97.5)		98.4 (95.0–99.7)	99.1 (96.6–99.9)
ICU patients with BSI	98.6 (95.8–99.7)	96.5 (93.1–98.5)	93.9 (89.3–97.0)		98.2 (94.6–99.7)	99.0 (96.4–99.9)

[a] BDG, (1→3)-β-D-glucan; Ag, antigen; Ab, antibody; CA, *Candida* antigen; CI, confidence interval; BSI, bloodstream infection; ICU, intensive care unit.
[b] Parameters are calculated separately for control group 1 (negative blood culture), control group 2 (bacteremia), and overall (control group 1 plus control group 2).
[c] Because the positive and negative predictive values ar dependent on the prevalence of the disease, they were calculated for three patient populations. The prevalence of candidemia during the last 10 years at our institution was 1.09% in all patients with a blood culture taken, 9.0% in all patients with bloodstream infection, and 10.1% in ICU patients with bloodstream infection.

BDG levels between *Candida* species showed no significant difference ($P = 0.296$). In contrast, patients with *Candida parapsilosis* and *Candida guilliermondii* fungemia had significantly lower mannan Ag levels than patients with fungemia caused by other *Candida* species ($P = 0.005$ and $P = 0.046$). In our study, *Candida parapsilosis* and *Candida guilliermondii* isolates were not detected by mannan Ag measurement. ICU patients and patients with *Candida* colonization had elevated mannan Ag levels. However, because the levels were below the cutoff, this would have had no clinical consequence.

Comparison of BDG with mannan Ag detection showed that the levels of both markers correlated in candidemia ($P < 0.001$), when the extreme outliers (BDG of >10,000 pg/ml; mannan Ag of >20,000 pg/ml) were excluded. There was no correlation between BDG ($P = 0.913$) or mannan Ag levels ($P = 0.608$) and the time-to-positivity of blood culture. In patients without candidemia, BDG levels increased with the time in the ICU ($P = 0.001$). This was not the case for mannan Ag ($P = 0.773$). However, the median BDG level of ICU patients was 20 pg/ml and therefore clinically not relevant.



	AUC (95%-CI)
BDG	0.925 (0.885-0.954)
Mannan-Ag	0.898 (0.854-0.932)
Mannan-Ab	0.673 (0.612-0.730)
	Optimized cut-off
BDG	≥ 70 pg/ml
Mannan-Ag	> 50 pg/ml
Mannan-Ab	≥ 15 AU/ml

FIG 1 ROC curves for BDG, mannan Ag, and mannan Ab. BDG shows the largest AUC; however, the difference between the AUC of BDG and that of mannan Ag is statistically not significant ($P = 0.361$). The AUCs of both BDG and mannan Ag are significantly larger than the AUC of mannan Ab ($P < 0.001$). According to the manufacturer, the mannan Ab detection should be used only in combination with mannan Ag detection. The manufacturer cutoffs were designed for a broad range of invasive fungal infections and therefore differ from the optimized cutoffs that were determined by us for candidemia alone. The criterion for the optimized cutoffs was the highest Youden index in the ROC analysis (indicated as circles). BDG, (1→3)-β-D-glucan; Ag, antigen; Ab, antibody; AUC, area under the ROC curve; CI, confidence interval; AU, antibody unit.

DISCUSSION

We conducted a retrospective study using the Fungitell assay, the Platelia *Candida* Ag and Ab Plus assays and the Cand-Tec latex agglutination test to evaluate BDG, mannan Ag, mannan Ab, and CA as biomarkers to aid in the diagnosis of candidemia.

Due to its low sensitivity (13.0%), the benefit of the Cand-Tec latex agglutination test in clinical routine is limited, and it should not be used on its own. Of the remaining biomarkers, BDG and the combination of mannan Ag plus Ab showed the highest sensitivities (87.5% and 89.3%, respectively). While BDG measurement produced a reasonable specificity of 85.5%, the specificity of mannan Ag plus Ab was only 63%. Responsible for this poor result is the integration of the antibody component, with a specificity of 65% when used exclusively. A similarly high sensitivity but with a specificity of 85% is achieved by combining BDG with mannan Ag. However, it is questionable if an increase in sensitivity of 1.8% justifies the extra costs of combination testing. Mannan Ag detection alone showed an excellent specificity of 97.5%, but this is offset by an intolerably low sensitivity of 58.9%. The ROC analysis revealed that by lowering the Platelia *Candida* Ag Plus cutoff from 125 pg/ml to 50 pg/ml, the sensitivity would increase to 69.6% without severely affecting the specificity (93.0%). In contrast, sensitivities and specificities of BDG measurement and measurement of the combination of mannan Ag plus Ab did not improve substantially by utilization of an optimized cutoff.

The lower sensitivity of mannan Ag detection is at least partially explained by our observation that *Candida parapsilosis* and *Candida guilliermondii* were not detected by the Platelia *Candida* Ag Plus assay. These two species comprised 12.5% of all *Candida* isolates. The sensitivity of mannan Ag in the other species was as follows: *Candida albicans*, 56%; *Candida glabrata*, 82%; and *Candida tropicalis*, 100%. Similar results were obtained with the predecessor of the current test. Fujita et al. described lower sensitivities for *Candida krusei* (0%), *Candida parapsilosis* (15%), *Candida guilliermondii* (27%), and *Candida glabrata* (36%) than for *Candida albicans* (78%) and *Candida tropicalis* (67%) (16). In an *in vitro* study, Rimek et al. analyzed the cross-reactivity of 63 fungi in the Platelia *Candida* Ag assay (17). Although they were using highly concentrated culture extracts, they were unable to detect *Candida krusei*, *Candida parapsilosis*, and a number of less frequently encountered *Candida* species. The reason for this nonhomogeneity in detection of different *Candida* species probably lies in the monoclonal EBCA-1 antibody, which was generated by immunization of rats with *Candida albicans*. This antibody binds to oligomannoside antigens that are abundant in the cell wall of *Candida albicans*, *Candida glabrata*, and *Candida tropicalis* but have been found to a lesser extent in *Candida krusei* and *Candida parapsilosis* (18). In addition, the variable antigen content may partially explain the missing correlation between the time-to-positivity of blood cultures and mannan Ag levels in our study. Existing antimycotic therapy, different sampling locations (catheter/peripheral vein), and the variable growth rates of *Candida* species (19) may further confound the time-to-positivity.

So far, there has been only one retrospective study using the novel Platelia *Candida* Ag and Ab Plus assays (13). In 21 patients with invasive candidiasis, Lunel et al. found an overall per patient sensitivity and specificity, respectively, of 61.9% and 43.3% for mannan Ag and 47.2% and 86.7% for mannan Ab (13). Because they followed a screening approach (multiple sera per patient) and used lower cutoffs (62.5 pg/ml and 5 AU/ml) and because a positive patient needed to have at least two positive sera, a direct comparison of their results and our own is not possible. However, the low specificity for the antigen and the high specificity for the an-

TABLE 4 BDG and mannan Ag levels in microbiological and clinical subgroups

Biomarker and subgroup[a]	Patient population[d]	Median biomarker concn (pg/ml)	*P* value	Significant difference between groups	Clinically relevant[b]
BDG					
Survivors vs nonsurvivors	Candidemia patients	347 vs 512	0.200	No	
Candida species in blood culture	Candidemia patients		0.296	No	
C. albicans vs other *Candida* spp.		370 vs 476	0.091	No	
C. glabrata vs other *Candida* spp.		650 vs 360	0.078	No	
Central venous catheter vs no catheter	Candidemia patients	429 vs 432	0.721	No	
Catheter infection vs no catheter infection	Candidemia patients	274 vs 380	0.716	No	
Antimycotic therapy vs no therapy	Candidemia patients	439 vs 401	0.608	No	
Candida colonization vs no colonization	Control groups 1 + 2	27 vs 19	0.385	No	
Patients in ICU vs non-ICU patients	Control groups 1 + 2	26 vs 20	0.261	No	
Major surgery vs no surgery[c]	Control groups 1 + 2	49 vs 20	0.009	Yes	No
Abdominal surgery vs no surgery[c]	Control groups 1 + 2	174 vs 20	0.010	Yes	Yes
Dialysis vs no dialysis	Control groups 1 + 2	52 vs 20	0.174	No	
Antibiotic therapy vs no therapy	Control groups 1 + 2	21 vs 18	0.857	No	
Bacteremia vs no bacteremia	Control groups 1 + 2	17 vs 21(3)	0.866	No	
Gram-positive vs Gram-negative bacteria	Bacteremia	17 vs 23	0.763	No	
Enterococcus faecalis vs other bacteria	Bacteremia	135 vs 15	0.040	Yes	Yes
Therapy with BL antibiotic vs no BL	Bacteremia	20 vs 21	0.903	No	
Mannan Ag					
Survivors vs nonsurvivors	Candidemia patients	172 vs 437	0.124	No	
Candida species in blood culture	Candidemia patients		0.008	Yes	Yes
C. parapsilosis vs other *Candida* spp.		11 vs 296	0.005	Yes	Yes
C. guilliermondii vs other *Candida* spp.		21 vs 295	0.046	Yes	Yes
C. tropicalis vs other *Candida* spp.		690 vs 172	0.061	No	
Central venous catheter vs no catheter	Candidemia patients	437 vs 37	0.044	Yes	Yes
Catheter infection vs no catheter infection	Candidemia patients	189 vs 64	0.414	No	
Antimycotic therapy vs no therapy	Candidemia patients	442 vs 163	0.309	No	
Candida colonization vs no colonization	Control groups 1 + 2	11 vs 0	0.001	Yes	No
Patients in ICU vs non-ICU patients	Control groups 1 + 2	13 vs 0	0.001	Yes	No
Major surgery vs no surgery[c]	Control groups 1 + 2	31 vs 0	0.001	Yes	No
Abdominal surgery vs no surgery[c]	Control groups 1 + 2	36 vs 0	0.001	Yes	No
Bacteremia vs no bacteremia	Control groups 1 + 2	0 vs 3	0.290	No	

[a] BDG, (1→3)-β-D-glucan; ICU, intensive care unit; BL, β-lactam; Ag, antigen.
[b] Differences were considered clinically not relevant if both subgroups had median antigen levels below the assay cutoff.
[c] Surgery during the 4 weeks prior to blood culture sampling.
[d] Control group 1, negative blood culture; control group 2, bacteremia.

tibody are surprising and in contrast to our results. Based on logistic regression analysis, Lunel et al. argued that the low antigen specificity was due to detection of mannan from patients with superficial candidiasis ($n = 4$). In our study, *Candida* species were cultured from samples of nonsterile sites in 40 of the control patients. Ten of these patients had a positive oral swab, and five patients suffered from thrush. However, none of these patients had a positive mannan Ag test, arguing against superficial candidiasis as a cause of elevated mannan Ag levels.

In candidemia patients with central venous catheters, mannan Ag levels were significantly elevated. This may be a consequence of a higher fungal load of the blood in these patients; alternatively, the blood may have been drawn through a *Candida*-colonized central venous line. The latter would point to an interesting question. Are different mannan Ag levels in blood drawn from a central venous catheter and a peripheral vein indicative of catheter infection? Further studies are needed to answer this question.

BDG has proved to be a sensitive biomarker for invasive fungal infections; however, because of its panfungal nature and various confounding factors, its specificity has always been an issue. While IVIG and albumin are definitely sources of false-positive results, other factors remain controversial (14). Among these possible confounding factors are bacteremia, certain antibiotics, *Candida* colonization, and treatment in an ICU. We compared the BDG levels of clinical subgroups and could not find significant differences between bacteremia versus no bacteremia, antibiotic therapy versus no antibiotic therapy, β-lactam therapy versus no β-lactam therapy, and *Candida* colonization versus no colonization. However, BDG levels were significantly elevated in patients with *Enterococcus faecalis* bacteremia and in the month after abdominal surgery. Of 10 patients with *Enterococcus faecalis* bacteremia, five had a positive BDG result. Because we were not able to detect relevant amounts of BDG in *Enterococcus faecalis* culture supernatants (data not shown), it is unclear whether *Enterococcus faecalis* itself is really the BDG source. One alternative explanation would be that *Enterococcus faecalis* bacteremia indicates only a gut barrier loss, resulting in an increased permeability for BDG. The same is the case for patients in the month after abdominal surgery. It is known that surgical exposure to sponges or gauze can lead to elevated BDG levels (20). However, baseline levels seem to be restored after 3 days (21), and the fact that these patients were still in the hospital several weeks after the operation suggests that they

suffered from potential complications like intestinal perforation with subsequent BDG translocation. Further studies are clearly needed to elucidate the impact of the various confounding factors on BDG measurement, always keeping in mind that BDG is not specific for *Candida* species but is also detected in other fungal infections like aspergillosis or pneumocystosis.

Besides the statistically significant differences, comparison of the analyte levels in the various subpopulations also showed some interesting trends. In particular, the higher BDG and mannan Ag levels in nonsurvivors point to a possible prognostic relevance of these biomarkers.

An approach to improve the performance of antigen assays is the combination with other diagnostic tools. One example is a study by Posteraro et al., who used BDG measurement together with the *Candida* score in 14 patients with invasive candidiasis and increased the sensitivity from 92.9% to 100%. However, this gain in sensitivity came at the price of a loss of specificity from 93.7% to 83.5% (22). The number of false positives in our study was particularly high in patients with bacteremia. Because there is evidence that PCT can be used to distinguish between bacteremia and candidemia (15), we decided to examine the effect of combining PCT with BDG in order to increase specificity. By doing so, specificity increased considerably, but the benefit of the combination did not outweigh the drastic loss of sensitivity. One reason for this was certainly that, in our study, PCT levels of bacteremia patients were not significantly higher than those of candidemia patients ($P = 0.36$).

Altogether, our data show that BDG, mannan Ag, and mannan Ab levels were significantly elevated in patients with candidemia. *Candida parapsilosis* and *Candida guilliermondii* were not detected by the Platelia *Candida* Ag Plus assay, reducing the sensitivity of mannan Ag measurement. Of the four tests compared, BDG and the combinations of BDG plus mannan Ag and of mannan Ag plus Ab showed the highest sensitivity. However, BDG with or without mannan Ag revealed a moderate specificity, and mannan Ag plus Ab had an unacceptably low specificity. The combination of PCT with BDG was of no essential benefit. BDG and mannan Ag seem to be the superior biomarkers for the diagnosis of candidemia, depending on whether a sensitivity-driven or specificity-driven approach is used.

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